

THE MORPHOLOGY AND THE HISTOLOGY
OF THE ALIMENTARY TRACT OF
LITTORINA IRRORATA (SAY)

By

Henry James Werner
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SECTION I

INTRODUCTION

Littorina irrorata (Say) was selected for this study for several reasons: First, *Littorina* is abundant within driving distance of the University of Maryland. Secondly, because the form, with few exceptions, has been practically ignored. A survey of the literature revealed the fact that the previous work which had been done on *Littorina* was either of an isolated nature or concerned with some specialized portion of the animal, no attempts having ever been made to examine the animal in its entirety. In this study an attempt is made to present the morphology and the histology of the alimentary tract of the snail - that which, in proper sequence, should have been the initial work done on the animal. An investigation such as this can serve as a spring board for subsequent cytological and physiological research on *Littorina*. Thirdly, an investigation of this nature has a practical transfer value of some importance. If the animal serves as a vehicle for sundry parasites of wild-life and then, ultimately, man, it seems quite apparent that a survey of the morphology and the normal histological aspects of the alimentary tract of the animal be available as a preliminary or prerequisite for pathological investigations.

The extent of the investigations on this form are as follows: Woodward (1942) reported on the development and the behavior of the nurse-cells of *Littorina*; Reinke (1912) wrote relative the development of the apyrene spermatozoa of this form; Newcombe, Phillips,

and Gould (1937) reported on the growth indices of *Littorina*; Green and Green (1932) reported on the shell growth of the same form.

The Littorinidae are prosobranch gastropods, and they are placed in the suborder Pectinibranchiata because these forms possess a pectinate gill or ctenidium which is the chief respiratory organ. They are placed in the division Taenioglossa by virtue of a taenioglossid radula with a characteristic tooth arrangement. The Littorinidae have a flat foot which is used in creeping and are therefore assigned to the subdivision Platyoda.

SECTION II

MATERIALS AND METHODS

The snails which were used in this study were all collected in the vicinity of Chesapeake Bay, specifically Solomons and Galesville, Maryland. Collection trips were made during the fall and the spring of 1946 and 1947. Littorinidae are to be found in community-like clusters on *Ammophila arenaria*, the beach grass on which the snails climb. The habitat of the snail is considerably varied, ranging in extremes from areas of beach grass and mud to areas which are characterized by debris and decaying organic material, representing a deviation from the normal habitat of *Littorina*. No significant histological differences were seen in animals collected from such widely different areas.

The snails which were used for sectioning ranged in length from fifteen to thirty-five millimeters. For micro-dissection, the larger forms were used primarily because of the greater ease in manual manipulation. For microscopic slide work, size was not a determining factor relative the selection of the animal. The snails which were brought into the laboratory were kept in gallon jugs and aquaria. These containers were covered with wire screening. A mixture of sea water and beach grass taken from the immediate vicinity of the area of collection was put into the containers. In this study it was considered neither desirable nor necessary to establish a laboratory colony. Initially, several attempts were made to keep large numbers of the snails on hand merely for the sake of convenience of

having the animals available at all times. However, the variations in temperature, the rapid, continual evaporation of the sea water, the subsequent high mortality rate - all these, coupled with the difficulties encountered in dissection served as factors which convinced one that the establishment of colonies should be dispensed with. Consequently, as more specimens for dissection and sectioning were needed, it was much more efficacious to return to the collecting areas for additional forms.

In addition to the preceding undesirable features, another difficulty was encountered in the laboratory. The snails were kept in containers into which, initially, a mixture of sand, mud, and sea water was placed. This procedure, at the time, seemed especially desirable as every attempt was being made to simulate, as nearly as possible, the natural habitat of the animal in an attempt to reduce the high mortality rate so prevalent whenever the animal is brought and kept in the laboratory.

It was subsequently discovered that sectioning became an impossibility because of sand particles which were ingested by the snails. To remedy the difficulty, the procedure of collecting snails from areas other than that of beach sand and keeping them in jugs devoid of either sand or mud was adopted. This solved, to a great extent, the difficulty of always finding small grains of sand in the alimentary tract. This method circumvented the necessity of using paraffined wire screen over the bottom of the aquarium as suggested by Carriker (1946). If, upon sectioning, it was found that the particular specimen still had particles of sand in its alimentary tract, no attempt was made to remove the debris. Instead, only that portion of the tract was used which was suitable for microscopic work, the

remainder being disregarded.

Before actual work could be carried out on the snails, some method had to be devised whereby the animals could be removed intact from their shells in a condition which was suitable to either subsequent morphological or histological studies.

The removal of the snail from its shell was actually one of the biggest obstacles encountered in the entire problem. In the beginning every suggestion, critical or casual, was tried. The literature was carefully reviewed in an attempt to find a method which would facilitate the removal of the snail from its shell. Snails were put in sea water in which temperatures ranged from four degrees centigrade to eighty degrees centigrade. This procedure was repeated, using instead fresh water. Next, acidic and basic media of various concentrations were substituted. All types of "relaxers" such as Epsom salts, for instance, were tried. Each of the methods proved to be of absolutely no avail. In fact, the antithesis was true. Exposing the snail to such varied treatments proved to be detrimental. The animals contracted even farther into their shells, thus making a normally difficult task practically an impossibility. As a last resort, the use of bone clippers was tried. It is true that the method seems crude, and one must admit that at first quite a number of snails were mutilated beyond use. However, in a relatively short while, facility was acquired; and with a minimum of difficulty and time, the animal was removed by means of the clippers without any signs of mutilation. By trial and error, it was soon discovered that the best method of denuding the animal was as follows: Insert the bone clippers laterally into the peristome. Upon contact with the clippers, the snail immediately withdraws even farther into the distal coiled portions of its shell. This serves as

a temporary advantage in that the entire anterior portion of the shell is then free to work in. By application of pressure on both sides of the peristome, the entire shell can be cracked open anteriorly and laterally. Thus the entire anterior end of the animal is exposed. With the aid of dissecting probes and forceps the animal can quite readily be removed from the coiled portion of its shell. Finally, it is necessary to cut the strong columellar muscle. This muscle serves as the animal's only direct means of attachment to the shell. Animals denuded in this fashion, requiring less time than one minute, were active and in every way suitable for further study.

Snails which were used for sectioning were sacrificed in one of two ways: first, by using the method as suggested by Drew (1936) of placing the animal, prior to fixation, in sea water, to which was added about one tenth of its volume of ethyl alcohol and turpentine (about ten cubic centimeters of turpentine to each one hundred cubic centimeters of alcohol) and leaving for several hours; secondly, by immersing the animal directly into the fixative at room temperature. Upon subsequent microscopic examination, very little, if any, autolysis of the cells of the alimentary tract was noticed when using either of the two methods. The latter, being the simpler of the two and equally as effective, was, of course, then used throughout the study. As a check, a few of the denuded animals were placed in ice-filled containers and kept there until the temperature ranged from five to two degrees centigrade. These animals were then put into the fixative which had been brought down to the same temperature. The comparative results, upon microscopic examination, verified the previous contention that little or no autolysis of cells occurred when the animals

were placed into the fixative which was kept at room temperature. Having found the latter method of no especial advantage, despite these temperature precautions to prevent autolysis, once again the simplest of the three methods was used.

For purposes of general fixation, Helly's mixture, Zenker's sublimate-bichromate mixture, and Bouin's picro-formol mixture were all tried. Helly's fluid was found to be the most satisfactory in that the granular cytoplasmic contents were more readily observed when fixed in this solution.

For purposes of dehydration, Helly's modified procedure of using the increasing concentrations of ethyl alcohol along with a ten percent alcoholic solution of iodine to seventy percent of ethyl alcohol, just enough to cause the latter to turn the color of port wine, was employed exclusively throughout the investigation. The alcoholic solution of iodine served to remove completely all traces of the mercuric salt which might still be present from the fixative.

Clearing was carried out in cedar oil. Infiltration and embedding were carried out in paraffin.

For microscopic examinations diverse types of sections and methods were used. First of all, longitudinal and cross serial sections were made of the entire denuded animal. Secondly, the complete alimentary tract was dissected from the animal and cross serial sections were made. Thirdly, for purposes of comparison, whenever any doubts prevailed, individual portions of the alimentary tract were isolated and run up separately. These were serially sectioned, cross or longitudinally, as the situation demanded.

For general routine staining, Harris' hematoxylin with an eosin counter stain was used. Approximately every hundredth section of tissue

was stained with iron hematoxylin. Using this method, one could easily verify the nuclear patterns as seen with the former stain technique. As this work was a histological study, no other specialized stains of a cytological nature were considered as being necessary. Tissue used in this phase of the work was cut at thicknesses ranging from five to three microns.

In the morphological phase of the work it was soon recognized that in order for micro-dissection to be carried out effectively, two conditions had to be met: first, the animal must be so conditioned that the threshold of its response be raised above the level of stimulation induced by ordinary dissecting methods; and secondly, it was essential to have a surface on which to fix the specimen, one which would permit light to be directed on the animal from all directions simultaneously, and one which would also hold the water which is applied almost continually during dissection.

To fulfill the first condition, snails were placed in various concentrations of magnesium sulphate and chloral hydrate in sea water. Narcotics such as ether, diluted concentrations of alcohol, and chloroform were found to be almost entirely useless. It was found that a mixture of four percent chloral hydrate mixture served a dual purpose: first it brought about a greater degree of relaxation; secondly, it resulted in one having a much quieter animal with which to work under the binocular microscope. Dissections were made in sea water which was brought from the immediate vicinity in which the snail was collected. To fulfill the second condition and to facilitate dissection, medium-sized petri dishes were partly filled with wax, and the animals were placed in these dishes during dissection. This served

as an admirable base of operation. One small insect pin was forced through the anterior propodium of the animal down into the wax base of the petri dish and another through the posterior extremity of the animal. Thus the animal was quite easily kept in a fixed position. Frequent applications of sea water and chloral hydrate acted as a local anesthesia and simultaneously kept the delicate tissues of the snail from drying out due to the intensity of the light which had to be kept in close proximity of the animal in order to carry on with dissections.

Insect pins were inserted into small oval-shaped corks. These, along with thin glass rods which were bent and drawn out as needed, served as dissecting instruments. To further facilitate dissection, as the living tissue is most translucent and therefore indistinct when viewed under the binocular microscope, solutions of carmine, India ink, methylene blue were injected into the alimentary tract of the snail. This was accomplished by means of glass rods which were drawn out to a diameter which was smaller than the oral aperture. The other ends were covered with small rubber pipette bulbs. This procedure was, of course, used only during the morphological phase of the work. Animals so treated were used periodically in the work of serial sections. The animals were cut at twenty microns and mounted unstained. The subsequent contrast brought out in vivid relief that portion of the alimentary tract which one wanted to follow.

SECTION III

GENERAL EXTERNAL ANATOMY

The denuded snail is spiralled in a sinistrally elongated fashion. Anteriorly, one finds the pale, truncated propodium or the anterior projection of the foot (Plates I and II). The dorsal surface of the propodium has two lateral ridges which extend from the ventral base of the tentacles to the dorsal mid-line concavity of the propodium. This depression is referred to as the propodial concavity (Plate II). The foot, situated ventrally to the propodium, is composed of a large mass of muscular tissue being covered by a general body epithelium. This muscle is continuous posteriorly with the columellar muscle. By means of the latter the animal is firmly adherent to its shell.

Normally the head is only slightly protruded. It is contiguous laterally with two short, muscular, circularly striated tentacles. A jet black eye is situated on a lateral projection of each tentacle. The eye is located approximately one third of the tentacular length from the distal tip of each tentacle.

In males a short translucent penis, almost comparable in length to a tentacle, projects from the lateral cephalic wall, posterior and ventral to the right tentacle. The penis is not visible from a dorsal view, being hidden by the dorsal mantle wall of the snail.

The mantle (Plates I and III) which completely encircles the anterior half of the animal is composed of a sheath of epithelium. It covers the entire cephalic hemocoel of the snail. The mantle extends posteriorly to the visceral region. It is relatively thin, as

evidenced by the fact that structures directly beneath it are partly visible under the binocular microscope. The mantle is bounded anteriorly with a relatively thick border. This rim of the mantle is slightly recurved to fit against the peristome of the shell.

The gonadal tissue is visible dorsally. It is a circumscribed mass of tissue which is located on the dorsal-lateral surface, left of the mid-region of the animal.

SECTION IV

MORPHOLOGY OF THE ALIMENTARY TRACT

The alimentary tract is divisible morphologically into the following regions: the buccal mass with its anterior opening, the mouth, the esophagus, the stomach, the intestine, and the rectum.

THE BUCCAL MASS

The buccal mass with all its associated structures is situated in the anterior part of the cephalic hemocoel. The cephalic hemocoel lies beneath the floor of the mantle cavity and rests on the muscular mass of the foot (Plate III). The cephalic hemocoel is distinctly separated from the posterior visceral mass by a wall of connective tissue. The hemocoel has been given diverse names such as cephalus, anterior sinus, etc. Lankester (1881), however, has shown that this structure, in which the buccal mass with its related structures are located, is a true hemocoel. The buccal mass is firmly suspended in this cavity by many short buccal muscles which extend outwardly in lateral, dorsal, and ventral directions. Thus the buccal mass itself is a relatively immovable structure.

The mouth resembles a small circular perforation in the anterior tip of the buccal mass. This aperture is bounded by the peristomal rim. The tongue-like projections of the odontophore are periodically seen protruding from it (Plate II). The oral aperture remains closed except during feeding periods. The mouth is rather difficult to locate because of the many pseudo oral openings formed by the numerous

invaginations and evaginations of the peristomal tissue which surround it. This is especially true whenever the animal is in a contracted stage. Posteriorly, the pharynx opens into the esophagus.

The buccal cavity of the pharynx encloses the complex feeding mechanism. Most prominent are the two odontophores (Plates II, V, VI, VII, VIII, and XII). These structures are triangularly shaped, dorso-ventrally compressed cartilaginous bodies. Their axes run in an anterior-posterior direction, parallel with the main axis of the animal. The odontophores, on their anterior-dorsal surface, serve as a constant support for the radular structure.

The radular structure is found in a median longitudinal sulcus situated between the odontophores on both their anterior and ventral surfaces (Plates VI and VII). The radular apparatus (Plates V, VI, VII, and XII) in conjunction with the odontophore, acts as the effective feeding mechanism. The radula slides continually and independently over the odontophore, providing in this fashion an effective cutting or rasping mechanism. Together, during feeding, these two structures are considerably everted. The radula is a long, narrow, and chitinous ribbon-like structure. It carries longitudinal rows of teeth, the arrangement of which is of taxonomic value. This structure has been called diverse names such as the basal membrane, the lingual plate, and the now accepted terminology, the radular ribbon (Sollas, 1907; Pruvot-Fol, 1926; and Peile, 1937).

The radular membrane inserts on the dorsal surface of the odontophoral cartilages. The radular membrane which lies in the dorsal longitudinal sulcus of the odontophores has lateral, strong, chitinous extensions which rise out of the sulcus and cover the odontophores

(Plates VI and VII). The radular ribbon extends anteriorly and dorsally from its insertion to the tip of the cartilages. It curves under the odontophore traveling posteriorly along the median sulcus of the ventral surface. The radula continues ventrally and posteriorly through a large mass of muscles, ventral to the esophagus, to the right of the mid-line of the cephalic hemocoel. It is a much convoluted structure which ends in a small, distal, ivory-colored bulb from which the teeth originate (Plate V).

ESOPHAGUS

Posteriorly the pharynx opens into a relatively small circular tube, the esophagus. Upon gross inspection the esophagus is considerably less muscular than the buccal mass. Throughout its entire length the esophagus is a comparatively flaccid structure. The walls of the esophagus are both thin and translucent. The walls are so thin that particles of undigested food are readily visible through the dissecting scope. In form the esophagus is a uniformly, cylindrical tube with its overall diameter remaining quite constant throughout its entire length (Plates IV, V, and XII).

The esophagus, from its opening in the posterior region of the pharynx, extends from this point throughout all of the cephalic hemocoel. The esophagus runs posteriorly through the central portion of the cephalic hemocoel. Its course is relatively straight until it is about ready to leave the hemocoel to enter the visceral mass.

Before one can continue with a discussion of the esophagus or any other portion of the anatomy of the alimentary tract, which is located in the visceral region of the animal, an explanation of how the terms "left" and "right," "ventral" and "dorsal" are going to be

used in the succeeding pages is desirable. It will be necessary to refer to Plate XII. In the adult condition, that portion of the animal posterior to the cephalic hemocoel has undergone considerable sinistral rotation, so much so that that portion of the animal which was embryologically dorsal in position is now actually ventral in position and vice versa. Similarly, that side of the animal which was embryologically the left half is, in the adult condition, the right half. The procedure has been adopted of designating areas of the visceral mass as they appeared in the embryonic condition. Thus the esophageal opening, for instance, in Plate XII, though it might seem to be located in the right dorsal portion of the animal, is, in actuality, located in the ventral left portion of the animal. All of the descriptive regional terminology of visceral contents will, in this paper, be given in terms of embryological positions.

The esophagus, at the juncture of the cephalic hemocoel and the visceral mass where sinistral torsion has occurred, bends sharply to the right, dips ventrally, pierces the connective tissue boundary of the two areas, and enters the visceral mass at the left anterior-lateral margin. Initially, the esophagus follows its ventral course; soon, however, it abruptly bends on itself, traverses the substance of the visceral mass and assumes a very superficial dorsal position. It maintains this level, travels posteriorly, and directly opens into the left lateral anterior boundary of the stomach (Plates V and XII).

Dissection of the esophagus directly posterior to the pharynx and throughout most of the cephalic hemocoel is made especially difficult by the presence of a large dorsal mucous gland. This gland is contiguous with the esophagus; in fact, the esophagus traverses the ventral

substance of the gland (Plates V and XX). Upon the slightest pressure or puncture, the extremely delicate walls of the dorsal gland rupture, and copious amounts of mucous-like material are emitted. In the region of the stomach, dissection is hampered by the fact that the texture and the consistency of the alimentary tract is practically identical with that of the surrounding material. In areas such as these, the injections, as described previously, were almost invaluable.

Paired salivary glands are present in the cephalic hemocoel (Plates IV, V, and XII). They are located on either side of the dorsal anterior part of the esophagus. These salivary glands are roughly oval or bean-shaped, slightly convoluted and ivory in color. They are discrete structures bounded by their own connective tissue capsule. Occasionally, the glands wrap themselves around the esophagus, being then closely adherent to the esophageal tract. The salivary glands open anteriorly into two minutely small ducts. These ducts proceed dorsally and anteriorly in a very torturous fashion to enter eventually into the lateral anterior portion of the buccal mass.

THE STOMACH

The stomach is a large irregularly shaped cavity situated in the dorsal anterior half of the visceral mass of the animal (Plates X, XI, and XII). The esophagus terminates, in a funnel-shaped fashion, along the left anterior dorsal boundary of the stomach. The intestine begins diametrically opposite the entrance of the esophagus by means of a longitudinal groove from the right anterior margin of the stomach at a level somewhat lower than that of the esophagus. These two openings, with the intervening connective tissue, serve as the anterior

boundary of the stomach.

Anteriorly and dorsally the stomach is in contact with the liver; dorsally and laterally the stomach is in contact with gonadal tissue. The large columellar muscle, by means of which the animal is strongly adherent to the shell, is located on the mid-ventral surface of the stomach.

The roof of the stomach is corrugated in appearance because of numerous low parallel ridges. From the floor of the stomach several folds project into the gastric cavity. Most conspicuous is the large gastric fold situated midway between the esophageal and the intestinal openings on the floor of the stomach. It extends along the entire length of the gastric floor (Plate X). The width of the gastric fold is greatest on the top; thus its upper edges overhang slightly. The shape of the fold in cross section can roughly be described as being T-shaped. The gastric fold is widest posteriorly, at which region it flares out laterally, gradually lowering and blending into the posterior and the posterior-lateral boundaries of the gastric cavity. In the mid-region of the stomach the gastric fold is much thinner and more obviously a dividing structure. Anteriorly, it again flares out laterally to blend into the anterior boundary of the stomach between the esophageal and the intestinal openings. This fold serves to divide the stomach incompletely into two separate longitudinal gutters. It serves as a physiological, not necessarily an anatomical, division of the stomach. In this way the gastric contents are kept at least partially and temporarily separated. Thus the process of digestion is facilitated in that it appears that the function of this gastric fold is to direct the flow of contents of the stomach and simultaneously prevent undue contamination of food.

Laterally and dorsally, the gastric walls are thrown up into many parallel ridges. The walls, too, therefore present a definite corrugated appearance.

On the right anterior margin of the stomach, directly lateral to the opening of the intestinal groove, the epithelial lining of the stomach is modified into a unique cuticular lining (Plates XI, XII, and XVIII). It is called the gastric shield. The shield is a slightly flexible, iridescent, somewhat irregular structure. The shield is broadest anteriorly, tapering off gradually at the posterior end. The lower edge of the gastric shield fits into the margin of one of the many lateral gastric folds. The latter serves as an anchorage for the gastric shield. At the anterior end of the gastric shield, the structure is bent inwardly on its dorsal surface toward the mid-line of the stomach, forming in this fashion an incomplete tube. The gastric shield is actually the entrance into the funnel-shaped intestinal groove, an opening which has a diameter somewhat smaller than that of the intestinal groove. The gastric contents, as they are propelled along the right side of the gastric chamber into the intestinal groove, undergo compression into fecal pellets by virtue of this shield. This process of compression of fecal material is continued along the intestinal route to the exterior.

THE INTESTINE

The intestine begins from the right anterior margin of the stomach (Plates X, XI, XII, and XX). It runs forward anteriorly from the stomach as a funnel-shaped intestinal groove at the same ventral level as the esophagus. The diameter of the intestinal groove gradually

narrows posteriorly until no distinction can be made between it and the intestine proper.

The course of the intestine is an anterior and gradually dorsal one which turns sharply to the right until the mid-line of the animal is reached. The intestine, at this point, is directly under the mantle cavity. It is separated from the cavity only by a layer of thin connective tissue. The convolutions of the intestine are readily visible beneath this very thin connective tissue layer. The intestine bends sharply and most acutely back on itself, traveling to the extreme left side of the animal. This convolution occurs at the same level which is dorsal to the preceding portion of the tract. Once again the intestinal tract takes a sharp turn; this time, however, the course of the tract is completely reversed - the tract now proceeding toward the right in the general direction of the mid-line and piercing the floor of the mantle cavity (Plate IX). This point of emergence of the intestinal tract from the visceral mass into the mantle cavity proper, plus a constantly recurring constriction in this region, serves as a morphological landmark for the beginning of the rectum.

THE RECTUM

Morphologically, the rectum is the terminal portion of the alimentary tract. One designates, as the rectum, that portion of the tract which lies along the posterior right margin of the mantle cavity and which is separated from the intestine proper by means of the constantly occurring constriction of which mention was made previously (Plates III and IX).

Histologically, as will be shown later, there is no difference between the intestine proper and what is herein designated as the

rectum. In length, the rectum is the shortest portion of the entire tract. In diameter, with the exception of the stomach, it is the largest.

The course of the rectum is a simple one, easily discernible, even without cutting the mantle covering. The rectum hugs the posterior boundary of the mantle cavity, curving slightly anteriorly to open eventually into the mantle cavity at the extreme right side. The end of the rectum terminates gradually into a small circular opening surrounded by a sphincter muscle. Grossly, the rectum is a large and flaccid structure with alternate areas of the tract being swollen and then normally contracted. The former is due entirely to the presence of the numerous fecal pellets located in this region.

SECTION V

HISTOLOGY OF THE ALIMENTARY TRACT

THE ESOPHAGUS

The esophagus is a thin-walled structure. Its walls are thrown up into internal folds of epithelium which line the lumen. This layer is supported externally by an outer, poorly developed circular layer of muscular fibers. The cell which is found in the epithelial lining is of the ciliated columnar variety. This type cell, in turn, is present in the esophagus in two distinct forms: first, the modified, highly distended glandular cell; secondly, the normal ciliated cell.

The glandular cells (Plate XVI) are actually modified columnar cells of the goblet variety. These have become immensely distended with mucus. These secreting cells are definitely larger than their precursors, the ciliated columnar cells. Heidermanns (1924) claims that these cells have cycles of both secretion and ciliation. While in the secretory stage, such cells lose their cilia. In the post-secretory stage a regeneration of the cilia occurs. One can say in support of Heidermanns' contention that in this investigation all cells which were observed in the pre- and post-secretory stages were ciliated, while those cells which were examined in the secretory stage were entirely devoid of any vestige of ciliation (Plates XV and XVI). These mucous cells are present throughout the entire esophageal tract, being present in various degrees of development. The cytoplasm of these cells assumes various transitional forms ranging from an alveolar type to a relatively clear reticular type. That portion of the cell nearest

the basement membrane contains fine promucin granules. Quite typically the secreting cells of the esophagus are to be found arranged in clusters or grouped on little islands (Plate XV). It is these cells which give the characteristic yellow color to the esophagus.

The other cellular constituent of the epithelial lining of the esophagus is the ciliated columnar cell. These cells are present in varying sizes and forms. They are, however, predominantly tall, thin, and irregular in contour. The ciliated cell is darkly stained. The bases of these cells rest on a delicate, often almost indistinguishable, basement membrane. The free ends of the cells are covered with an abundant amount of cilia. The nuclei are small, oval in shape, highly chromatic, and are stratified into various levels. The normal position of the nuclei is a basal one; frequently, they are medially located. The result of such a staggering of the nuclei is the appearance of pseudo stratification. These ciliated cells are not rectangularly columnar as one would expect. They are, instead, quite disproportionately tall and thin and most irregular. All this is due to the lateral compression which they are subjected to by the numerous distended mucous cells, the various contortions assumed by the esophageal tract, and the inward projections of the epithelial lining. The cytoplasm in all of these cells is normally finely but densely granular.

The muscular structure of the esophagus consists of definitely circularly arranged layers of muscle fibers. These fibers are generally disposed in a parallel fashion, occasionally irregularly interwoven. The numerous nuclei are arranged, more or less, with their axes along the main direction of the muscle fibers. The muscular layer is, in turn, covered by a connective tissue sheath which surrounds not only this region, but all of the alimentary tract. In

cross section (Plate XX) the lumen of the esophagus is divided by virtue of epithelial folds which project inwardly. These invaginations are comparable to the so-called villi of the higher forms.

THE STOMACH

The stomach, in cross section (Plate XX), appears to be a greatly enlarged esophagus. Epithelial folds are present but most irregular in both size and arrangement. The inner walls of the stomach are lined with ciliated epithelium which is quite similar to that which is found in the esophagus; in fact, similar to that which will be found to be present in the remainder of the various divisions of the alimentary tract (Plate XVII). The cells are all of the tall columnar variety, and in all instances except one, they are densely ciliated. Fine granules are distributed in the ground substance of the cells, being denser toward the distal end of the cell. The peri-nuclear regions of the cell appear to be relatively vacuolated. These cells rest on a delicate, though constantly preceptible, basement membrane. The rounded nuclei of the cells are basally situated, one third of the distance from the basement membrane. Occasionally, the nuclei are staggered in position, thus presenting again, to the casual observer, the appearance of pseudo stratification.

The nuclei of the columnar cells are sharply marked off from the cytoplasm by a distinct nuclear membrane. They are usually poor in chromatin, but prominent nucleoli are readily observed throughout all or most of the nuclei.

Gland cells, for all intent and purpose, are not present in this area of the alimentary tract.

The muscular layer of the stomach is very poorly developed.

There is instead a vague, loosely arranged type of alveolar connective tissue. In the right anterior portion of the stomach, in the region of the intestinal opening, the epithelium develops a cuticular lining known as the gastric shield (Plates XI, XII, and XVIII). The epithelial cells which support the gastric shield are invariably much taller and narrower than the adjacent columnar cells of the epithelial lining. Obviously, no cilia are present beneath this shield. This area, along with the modified glandular portions of the esophageal tract, is the only part of the entire alimentary tract which is lacking in ciliated cells. The cells in the region of the gastric shield take a much lighter stain than the neighboring cells of the gastric epithelium.

THE INTESTINE

In cross section, the intestine presents a very constant and typical picture (Plates XXI and XXII). The epithelial tract is highly convoluted and thrown into numerous folds. Typical intestinal villi are present as the result of the numerous invaginations of the epithelial lining. Fecal pellets can be seen both macroscopically and microscopically throughout the entire portion of the intestinal tract. The typical cell present in the intestinal tract is again the tall, ciliated, columnar cell. In this instance, the ciliation is considerably longer than in any other portion of the alimentary tract exclusive of the rectum.

Because of the numerous intestinal villi which are present, the cells have undergone a great amount of compression. This results in the nuclei appearing to be located at various levels. This again results in a true picture of pseudo stratified columnar epithelium.

The nuclei are generally basal in position and when found basally situated, they are ovoid in shape. When the nuclei are situated in the distal third of the cells, in the vicinity of the free ends, where there is an increased amount of compression, the nuclei are elongated in the direction of the main axes of the cells. The ground substance of the cells is finely granular and distinct nucleoli are visible throughout.

The cytoplasm in the proximal portions of the cells usually has a finely granular texture. However, in the remainder of the cell, specifically, the distal or the free portion, varying quantities and sizes of secretion granules are much in evidence. The degree and the distribution of the secretory granules apparently corresponds to the phase which the cells are in at the time of observation. In the final phase of secretion, the entire distal end of the cell may be cast off (Plate XXII). The intestine has for its principal support a very thin layer of circular muscular tissue.

THE RECTUM

Histologically, one can find no unique differentiating features for this terminal portion of the alimentary tract (Plate XXIII). This region is in every instance, histologically comparable to the before-described intestinal tract. One uses the term "rectum" because it designates, as a morphological entity, that portion of the alimentary tract which lies in the mantle cavity. Histologically, there is no necessity for a division of the intestine proper from the terminal portion of the tract.

SECTION VI

DISCUSSION

Studies of a nature similar to the one undertaken in this manuscript are difficult to find, the reason being that the aims were always different. Two works are worth mentioning. Carriker and Bilstad (1946) reported on the histology of the alimentary tract of Lymnaea stagnalis appressa (Say). The anatomical bases of that snail had been worked out prior to their report. The morphology of the alimentary tract of L. s. appressa (Say) is entirely different from that reported here on *Littorina* - different in that such divisions as the crop, gizzard, pylorus pre-esophagus and postesophagus are not apparent in *Littorina*. Carriker and Bilstad, in their exhaustive histological investigations, were concerned primarily with the type cells present rather than a histological comparison of the various divisions of the alimentary tract. They reach a similar conclusion; that is, with the exception of the crop (which is not present in *Littorina*) the alimentary tract is completely ciliated. Carriker and Bilstad have found many more types of cells than those reported prevalent in *Littorina*. Their work was of especial interest in that many suggested procedures such as laboratory colonies, extraction aids, and technique hints were mentioned. Most suggestions were tried but discarded, as mentioned in Materials and Methods, because simpler techniques were developed.

Most similar to the work undertaken in this manuscript is that of Magruder (1935) on the Anatomy of the Fresh Water Prosobranchiate

Gastropod, Pleurocera canaliculatum undulatum (Say). In general, the morphological and the histological findings of the Gastropod agreed with that found in Littorina. Outstanding differences are: first, the salivary glands as described by Magruder were long filamentous structures while those of Littorina were circumscribed ovoid bodies; secondly, the discovery of a crystalline style and associated structures as reported by Magruder were not to be found in Littorina.

SECTION VII

SUMMARY

Animals were removed from shells by means of bone clippers and dissecting probes. Helly's mixture and modified procedure were used for fixation and dehydration. Clearing was carried out in cedar oil; infiltration and embedding, in paraffin.

Morphologically, the alimentary tract of Littorina irrorata (Say) is divisible into the following regions: the mouth (which opens into the anterior tip of the buccal mass), the buccal mass and associated structures, the esophagus (which opens into the left anterior region of the stomach), the stomach, the intestine (which begins at the right anterior margin of the stomach and proceeds anteriorly), the intestine, and the rectum.

Histologically, the entire epithelial lining of the alimentary system is composed of ciliated columnar cells. In many instances, the epithelial lining, because of a stratification of the nuclei, brought about by the villi and convolutions, appears to be of the stratified columnar variety. Such is not the case as all cells rest on a delicate basement membrane.

Highly distended clusters of glandular cells of the goblet variety are found in the tract. These are especially prominent in the esophagus. Cytoplasm in most of the columnar cells is normally finely but densely granular. Nuclei are found to be usually basally situated and nucleoli are visible. Nuclei in the basal portions of the cells are ovoid; those in the free ends of the cells are often

compressed in the direction of the long axis of the cell.

The musculature of the alimentary system is composed of circularly arranged fibers present in all regions with the exception of the stomach in which region one finds a loosely arranged type of alveolar connective tissue.

SECTION VIII

PLATES AND DESCRIPTIONS

PLATE I

EXPLANATION OF PLATE

Camera lucida x 7

Dorsal view of denuded

snail in normal position

Key to plate:

1. Propodium
2. Peristomal rim
3. Tentacles
4. Mantle margin
5. Mantle
6. Gonad

PLATE 1

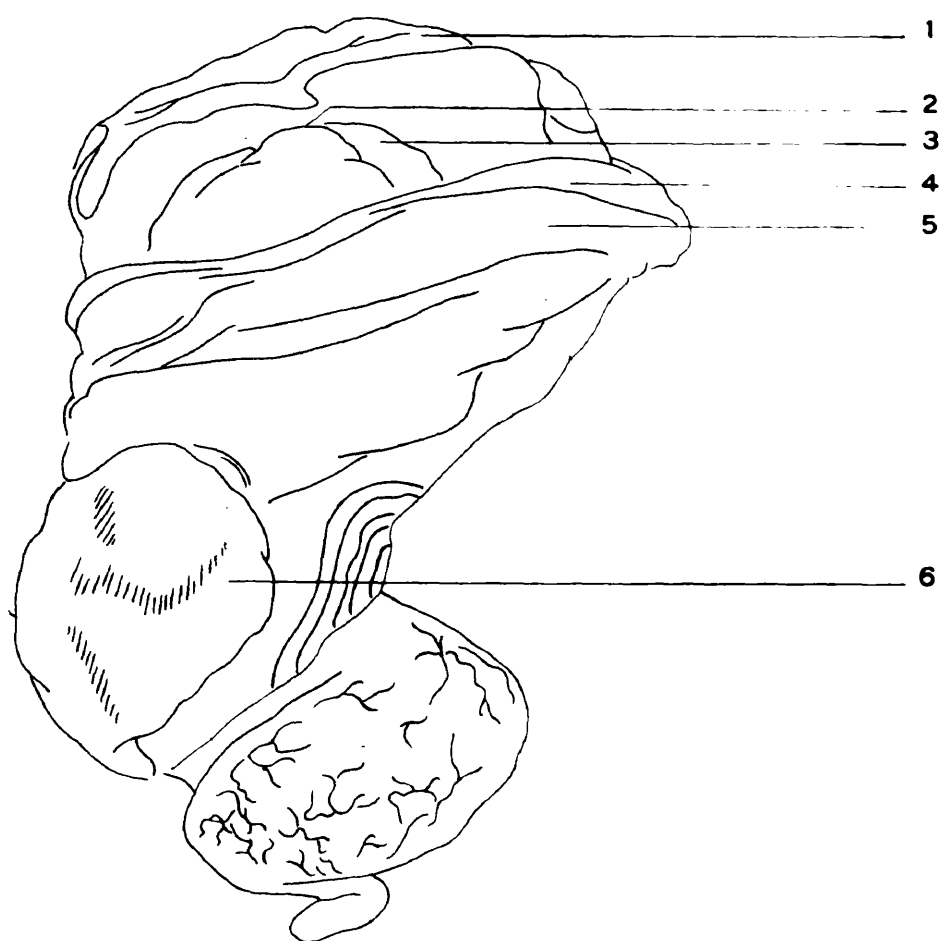


PLATE 2

EXPLANATION OF PLATE

Camera lucida x 7

Dorsal view showing the dorsal-anterior surface of the extended propodium. The mantle has been forced posteriorly completely exposing the tentacles.

Key to plate:

- 7. Anterior surface of propodium
- 8. Propodial concavity
- 9. Odontophores projecting from oral aperture
- 10. Peristomal rim
- 11. Propodial ridge
- 12. Tentacles

PLATE 2

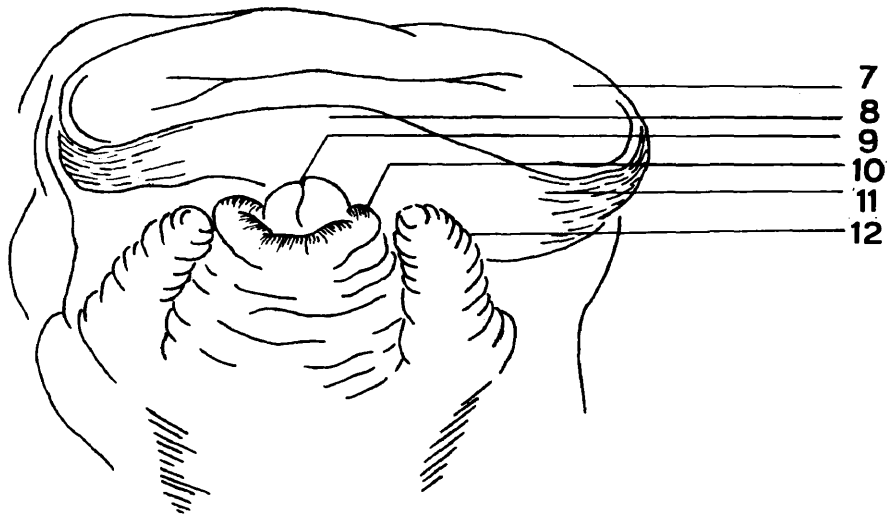


PLATE 3

EXPLANATION OF PLATE

Camera lucida x 14

Dorsal view. Superficial dissection.

Mantle has been cut sagittally.

The cut edges spread laterad.

All of the mantle cavity now

visible. The anal opening and

the rectum are visible along

the right and posterior bound-

aries of the mantle cavity.

Key to plate:

13. Roof of cephalic hemocoel

14. Anal opening

15. Rectum

16. Cut edge of mantle

PLATE 3

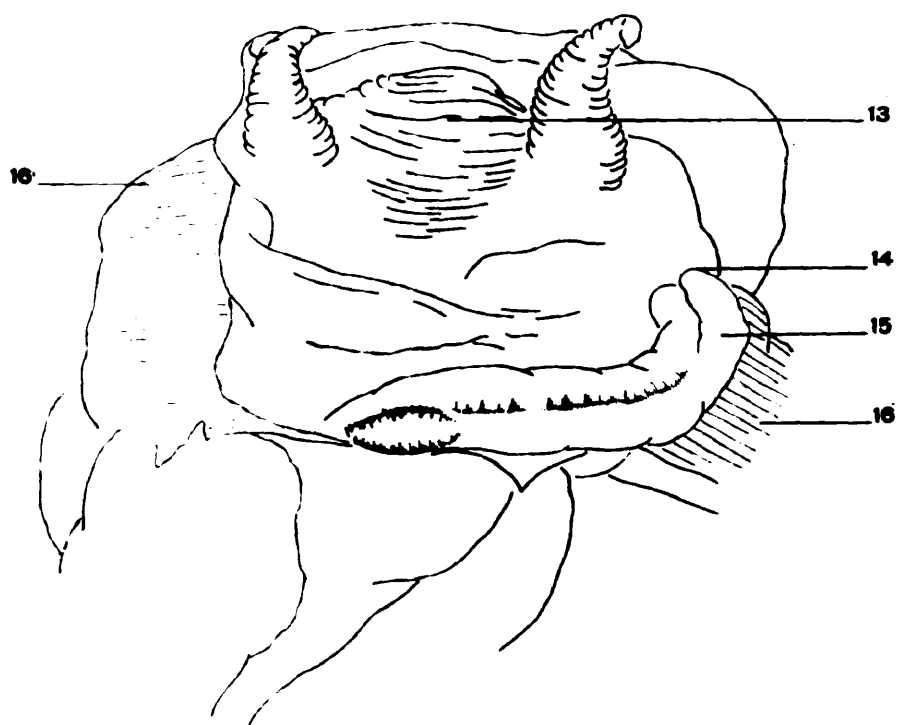


PLATE 4

EXPLANATION OF PLATE

Camera lucida x 7

Dorsal view. Superficial dissection of the cephalic hemocoel. The roof has been cut sagittally, spread laterad. Portions of the buccal mass, esophagus, and salivary glands are exposed.

Key to plate:

- 17. Median sulcus
- 18. Cut edge of cephalic hemocoel
which has been spread laterad
- 19. Salivary duct
- 20. Salivary gland
- 30. Mucous gland of mantle
- 40. Esophagus

PLATE 4

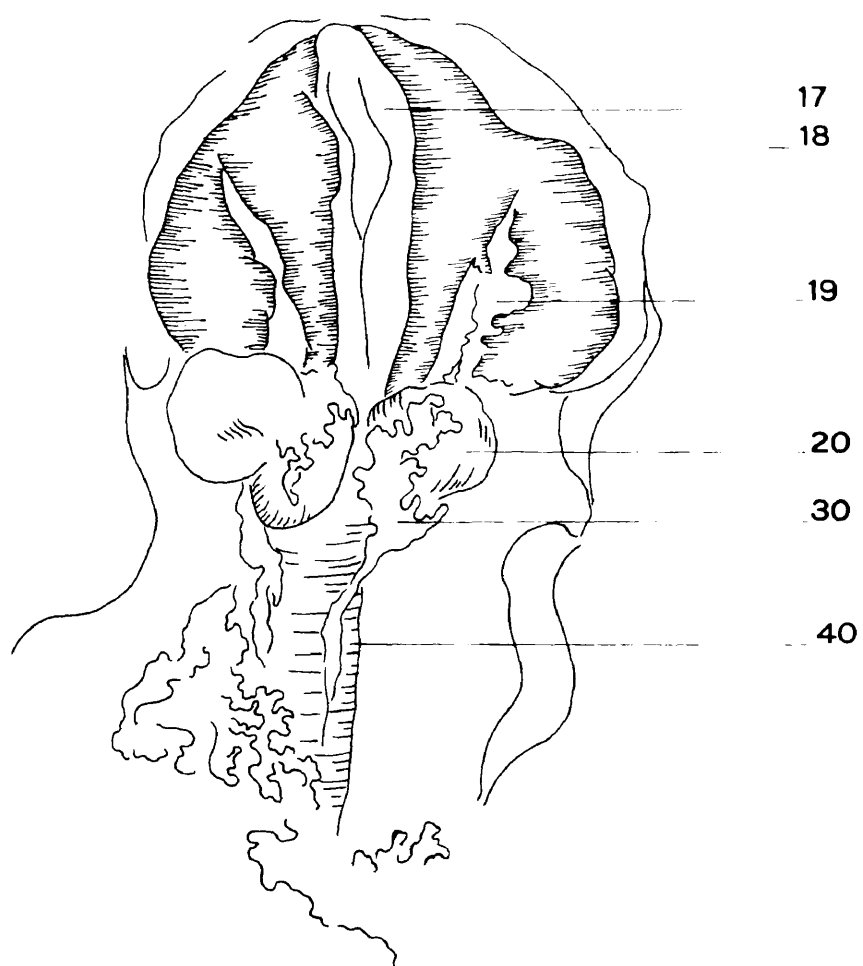


PLATE 5

EXPLANATION OF PLATE

Camera lucida x 7

Dorsal view. Deep dissection exposing the alimentary tract to the point where it is about to leave the cephalic hemocoel. The most anterior portion of the tract normally lies in the mid-line dorsad. In this plate, it has been displaced laterad to demonstrate the median sulcus, radula, and a dorsal view of the odontophore.

Key to plate:

- 17. Cartilage of odontophore
- 18. Median sulcus
- 19. Radula
- 20. Radular sac
- 30. Distal bulb of radula
- 40. Dorsal mucous gland of the
mantle cavity
- 50. Esophagus

PLATE 5

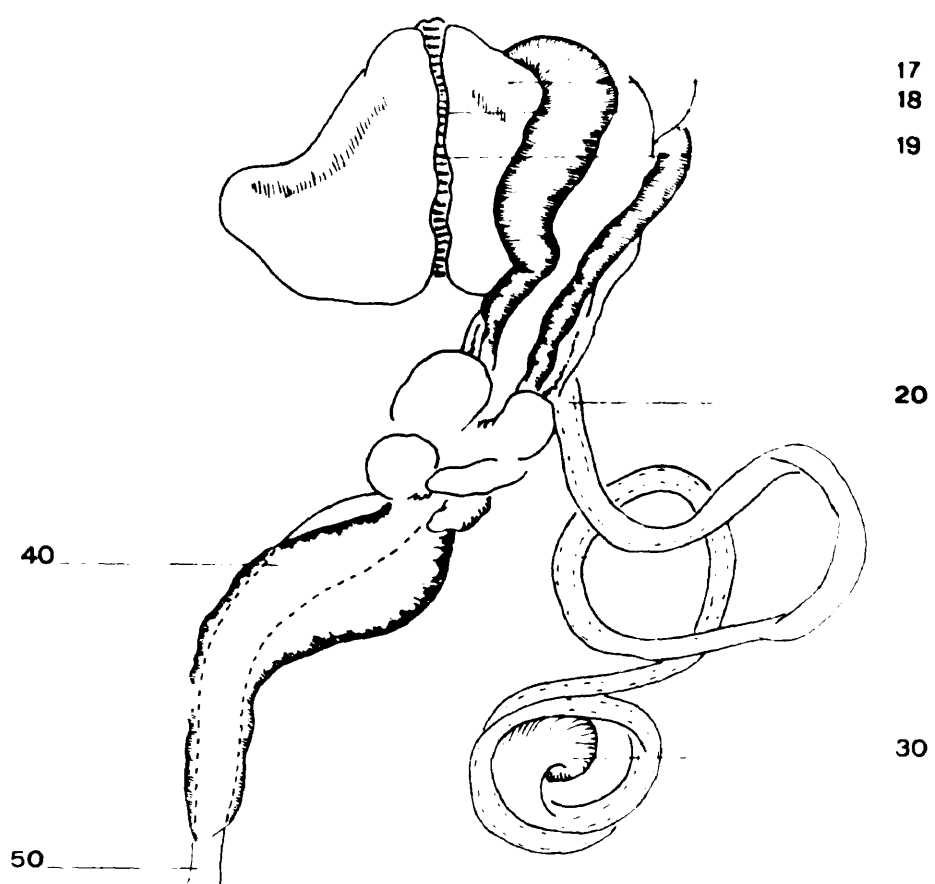


PLATE 6

EXPLANATION OF PLATE

Camera lucida x 14

Ventral view of the radula demonstrating the lateral flare of radula at the region of its insertion

Key to plate:

6. Insertion of the radula
7. Radula traversing the ventral median sulcus
8. Radular shield
9. Radular membrane

PLATE 6

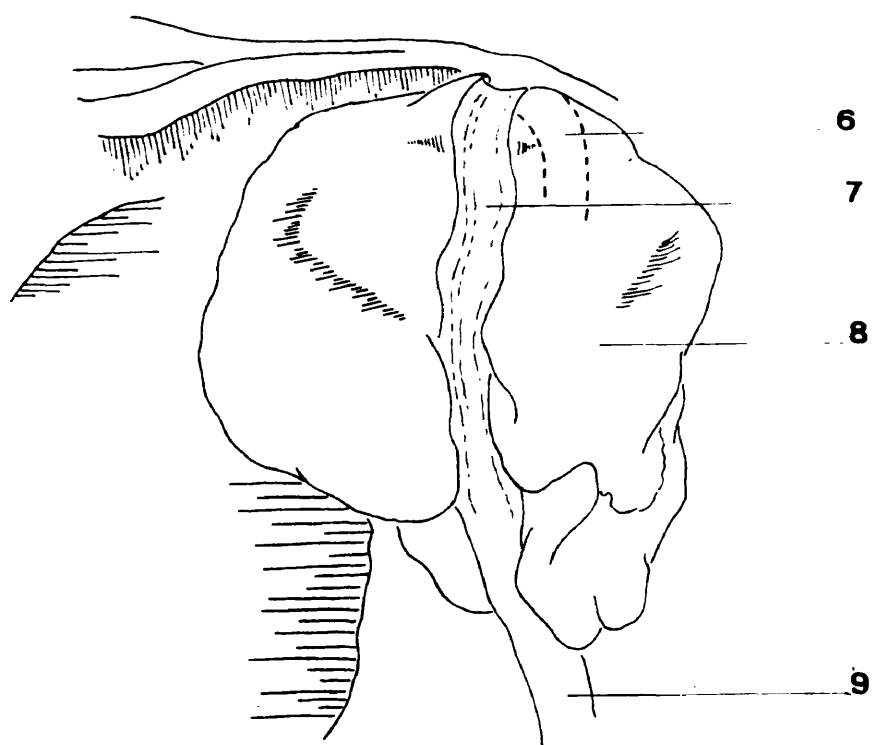


PLATE 7

EXPLANATION OF PLATE

Camera lucida x 7

Ventral view showing the tips of
the cartilaginous odontophore
projecting anteriorly from the
radular shield

Key to plate:

- 12. Odontophore
- 13. Radular shield
- 14. Radular sheath

PLATE 7

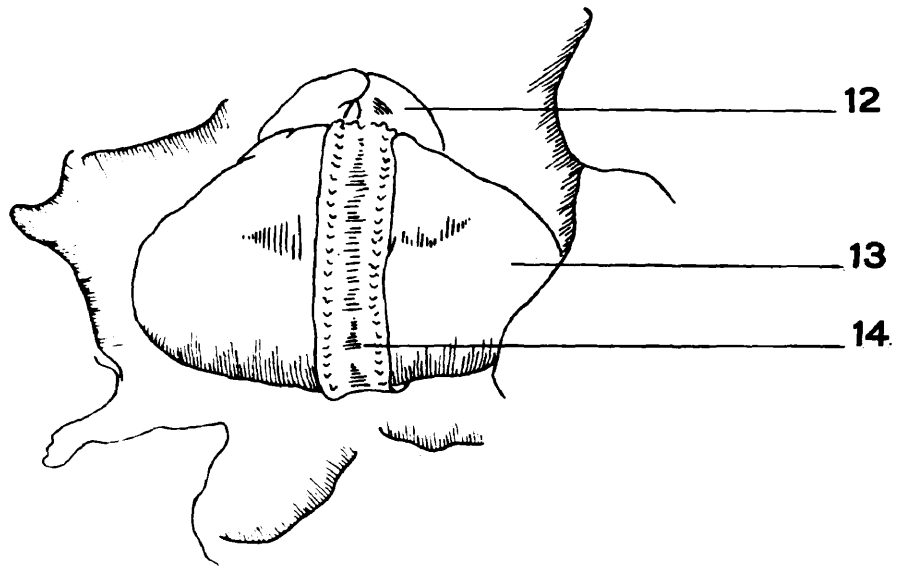


PLATE 8

EXPLANATION OF PLATE

Camera lucida x 14

Dorsal view. Deep dissection exposing the odontophores

Key to plate:

- 70. The thin connective covering
which has spread laterad
- 80. Two odontophores with median
sulcus between them
- 90. Cut edge of the mantle

PLATE 8

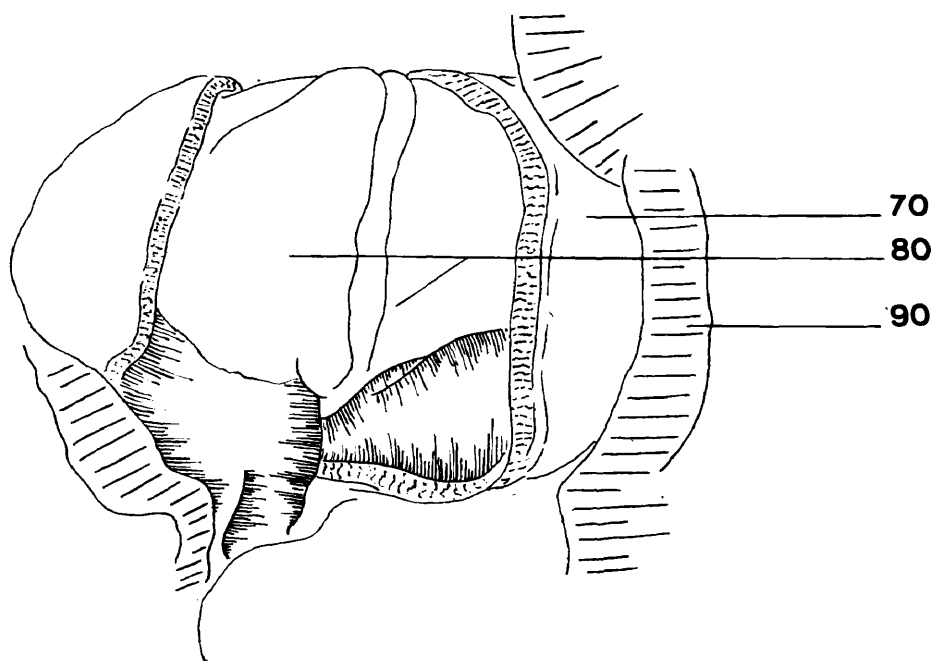


PLATE 9

EXPLANATION OF PLATE

Camera lucida x 14

Dorsal view. Superficial dissection of the posterior portion of the mantle cavity, exposing the highly convoluted intestinal tract and the rectum

Key to plate:

70. The constantly occurring constriction which serves as the morphological landmark for the beginning of the rectum
80. The intestinal tract proper

PLATE 9

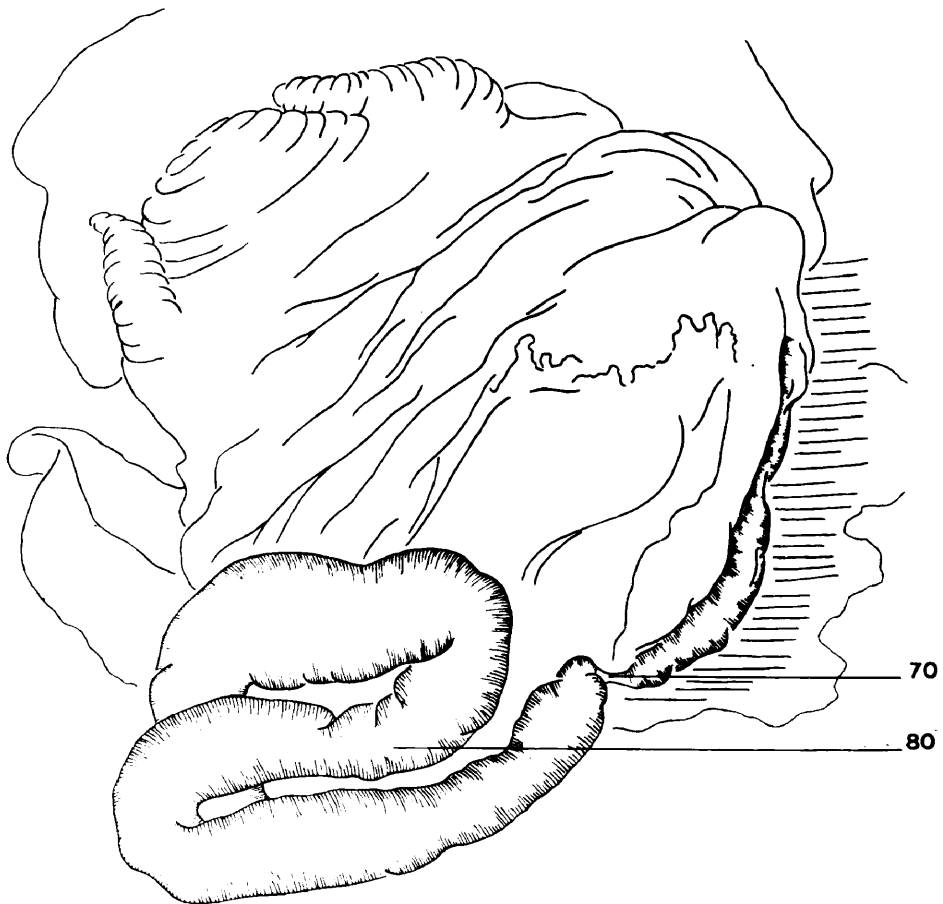


PLATE 10

EXPLANATION OF PLATE

Camera lucida x 14

Dorsal view. Deep dissection of
the stomach showing the corrugated appearance of the stomach and the esophageal and intestinal openings

Key to plate:

1. Lateral corrugated walls of the stomach with their many low parallel ridges
2. The median gastric fold of the stomach floor
3. Intestinal opening
4. Intestinal groove
5. Esophageal opening
6. Superficial lateral course of the esophagus

PLATE 10

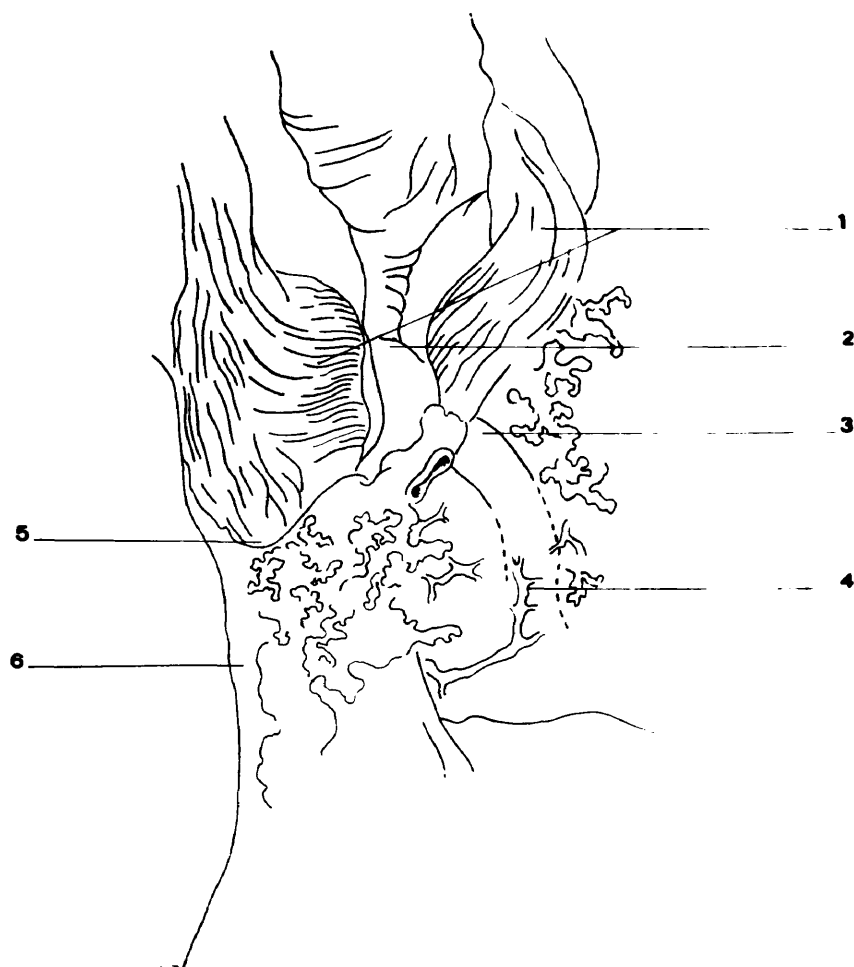


PLATE 11

EXPLANATION OF PLATE

Camera lucida x 14

Dorsal view. Deep dissection
made anteriorly to the dis-
section as illustrated in
Plate 10

Key to plate:

7. Anterior end of intestinal
groove which would join with
the blind ventral end of the
intestinal tract of Plate 9
8. Opening of the groove into
stomach
9. The lateral gastric ridge
which serves as an anchorage
for the gastric shield
10. Gastric shield
11. Cut lateral edge of stomach
12. Cut medial edge of stomach

PLATE II

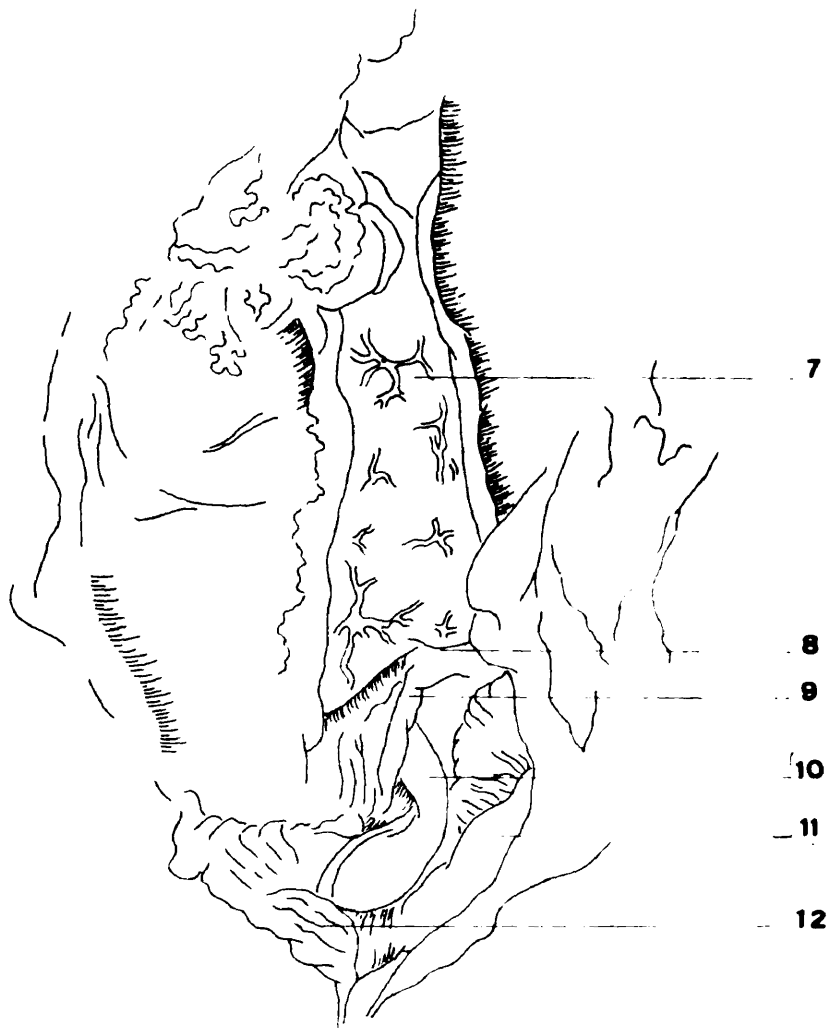


PLATE 12

EXPLANATION OF PLATE

Camera lucida x 7

Dorsal view of the animal which
has been forcibly expanded to
facilitate putting in a sche-
matic drawing depicting the
entire alimentary tract as it
is found in the animal

Key to plate:

- 13. Tentacle
- 14. Odontophore
- 15. Median sulcus
- 16. Salivary duct
- 17. Salivary gland
- 18. Radula
- 19. Distal bulb of radula
- 20. Dorsal mucous gland
- 30. Esophageal opening
- 40. Median gastric fold in floor
of stomach
- 50. Gastric shield
- 60. Lateral gastric fold which
serves as anchorage for the
gastric shield
- 70. Intestinal opening
- 80. Intestine proper

PLATE 12



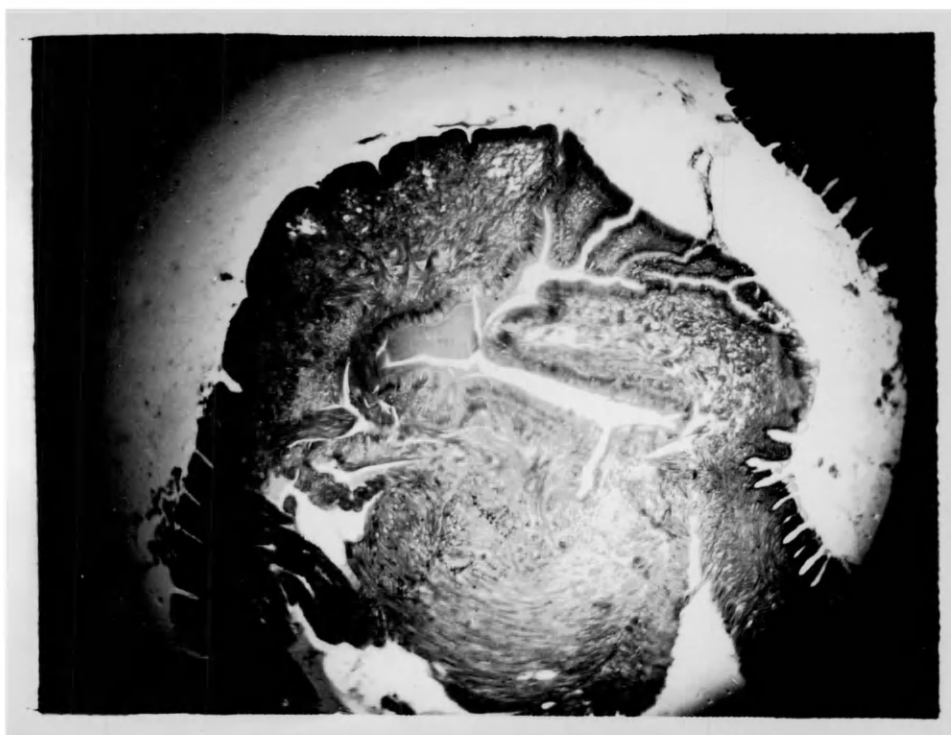


PLATE 14

EXPLANATION OF PLATE

Longitudinal section of the esophagus x 150

Section through the esophagus showing the openings of the paired salivary glands are two lateral diverticulae from the most anterior portion of the tract. The paired salivary glands are seen posteriorly.

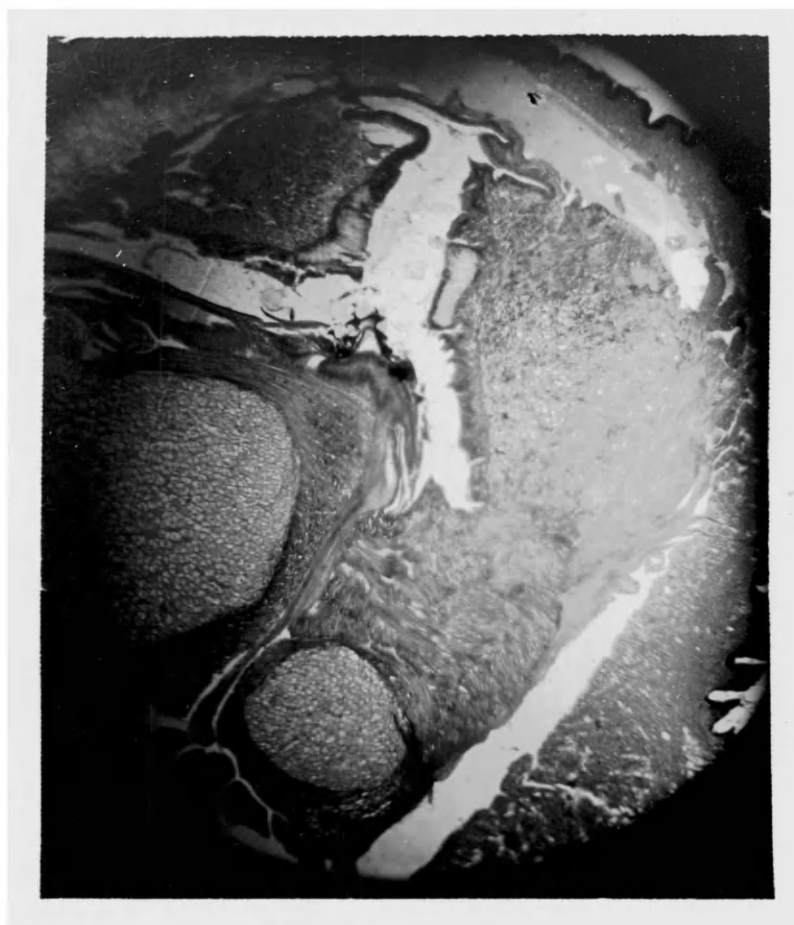


PLATE 15

EXPLANATION OF PLATE

Longitudinal section of the esophagus x 300

Section of the esophagus taken from the mid-region of the cephalic hemocoel. Anteriorly, two clusters of glandular cells are visible. Those on the left side of the tract are in the process of secretion. Mucin granules are being extruded into the lumen of the esophagus. Posteriorly the lumen narrows to its normal size being flanked on either side by the columnar ciliated cell of the esophagus.

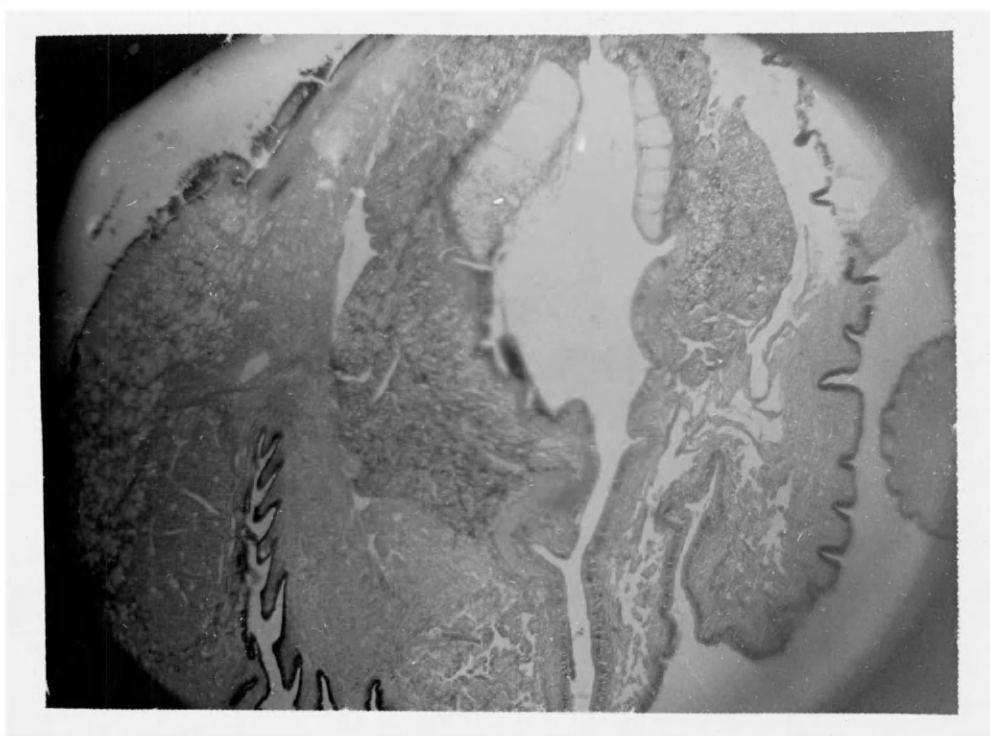


PLATE 16

EXPLANATION OF PLATE

Cross section through the esoph-
agus x 950

Section taken through the esophageal wall which shows both types of cells present in this region of the alimentary tract. In the dorsal portion of the plate greatly distended mucous cells are visible. Cilia are absent in these cells. Cytoplasm is of an alveolar nature. On the right margin, the usual tall, thin irregular ciliated cells are visible. Cilia are most abundant in these cells. The small nuclei are staggered, giving the appearance of pseudo stratification. Underneath these cells a delicate layer of circularly arranged muscle fibers are evident.



PLATE 17

EXPLANATION OF PLATE

Cross section of the stomach
wall x 950

Section through the stomach showing the tall, ciliated columnar cells. Ciliation is very dense and regular in this portion of the tract. A delicate basement membrane is visible. The nuclei of the cells are basally arranged except at the lower right margin where there is a considerable staggering, thus giving the appearance of pseudo stratification. No muscular layer is evident; instead the loosely arranged connective tissue is present.

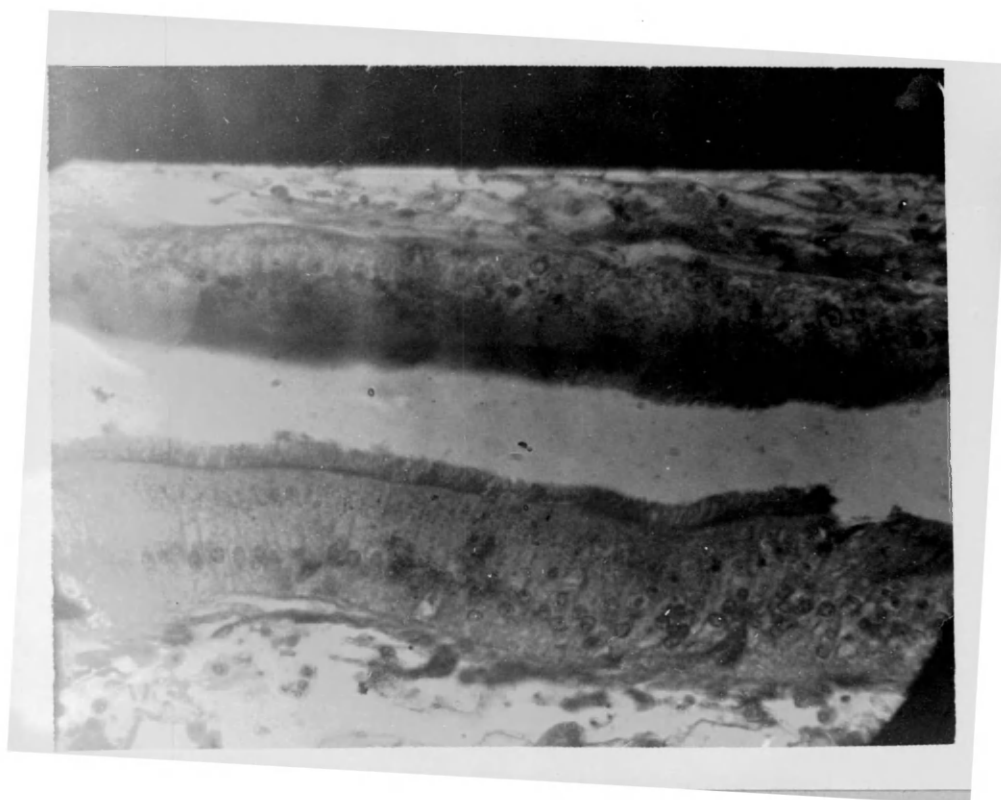


PLATE 18

EXPLANATION OF PLATE

Cross section x 300

Section taken through that region of the stomach in the vicinity of the intestinal groove where the epithelial cells develop a cuticular lining known as the gastric shield. The epithelial cells which support this structure are taller than the adjacent cells and they take a much lighter stain than the neighboring cells. Cilia are present on the ordinary cells in the left margin of the plate. A basement membrane is visible along the left margin of the cells.

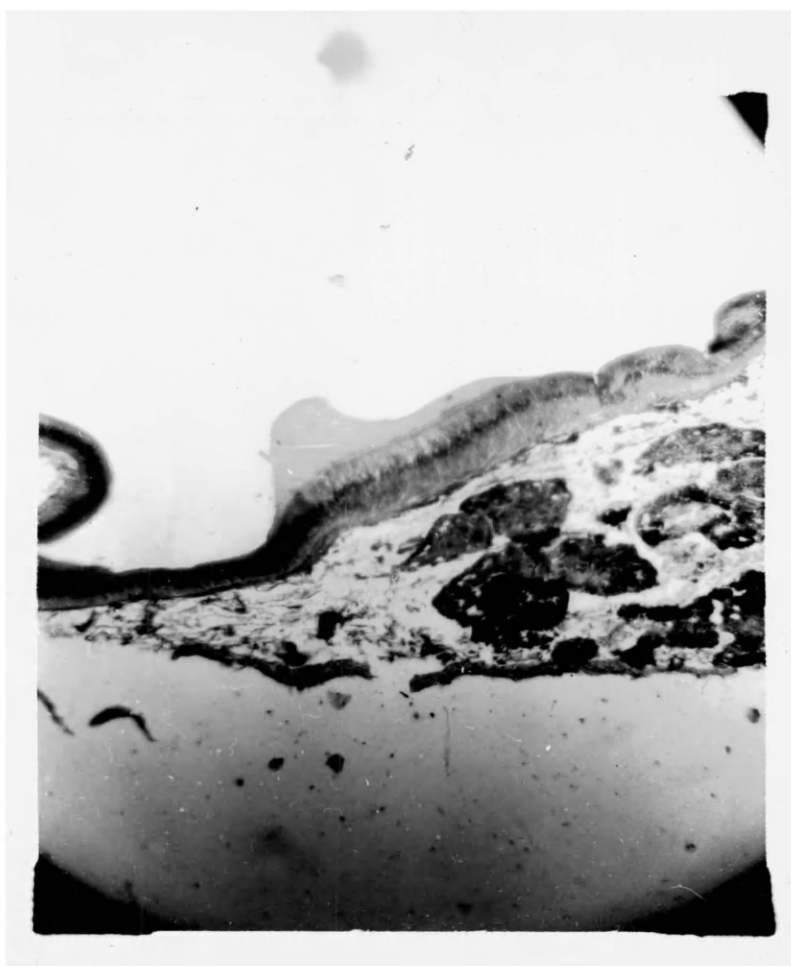


PLATE 19

EXPLANATION OF PLATE

Longitudinal section x 12.5

Section taken at this low mag-

nification to demonstrate the

corrugated appearance of the

interior of the stomach. Most

apparent are the numerous low

parallel ridges.

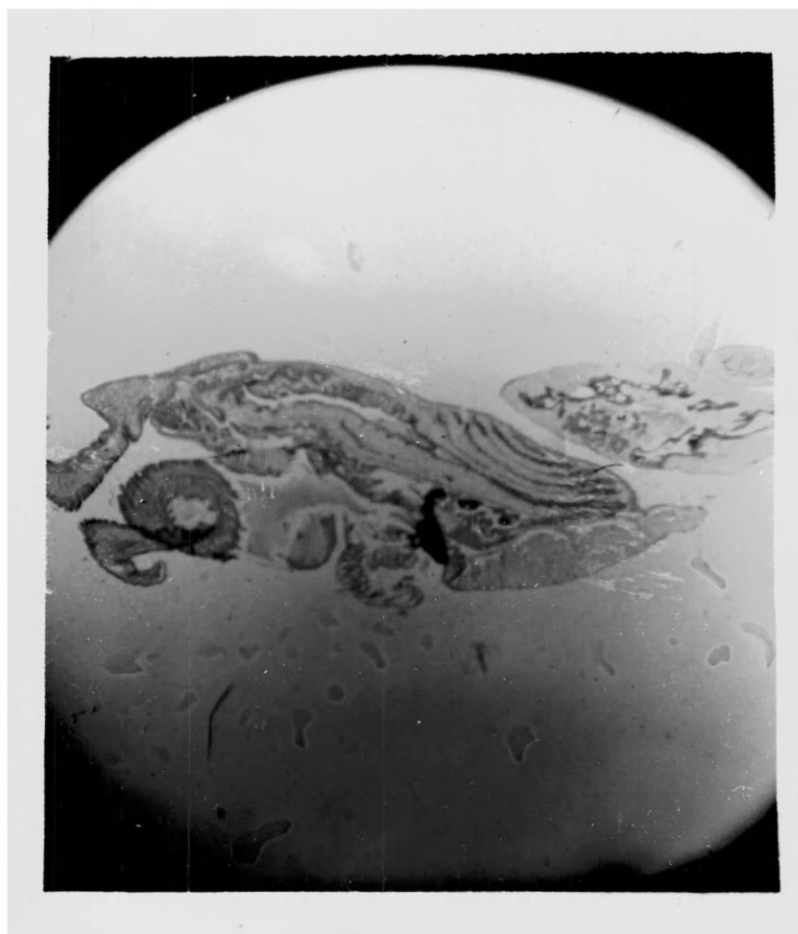


PLATE 20

EXPLANATION OF PLATE

Cross section x 300

Section taken through the visceral hump showing the relationship of the centrally situated stomach and the openings of the esophagus and intestine. The esophageal opening is situated on the left (embryologically speaking) side of the stomach. The intestinal groove, with fecal pellets, is situated on the right side of the stomach.



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PLATE 21

EXPLANATION OF PLATE

Cross section of the intestine x 300

Complete cross sectional view of the intestinal tract showing the generalized arrangement of the intestinal villi, the ciliation, and the fecal pellets. Notice that the intestinal villi are much more numerous and higher than those found in the rectum. The cilia are much more prominent than that which is found in any other portion of the tract.

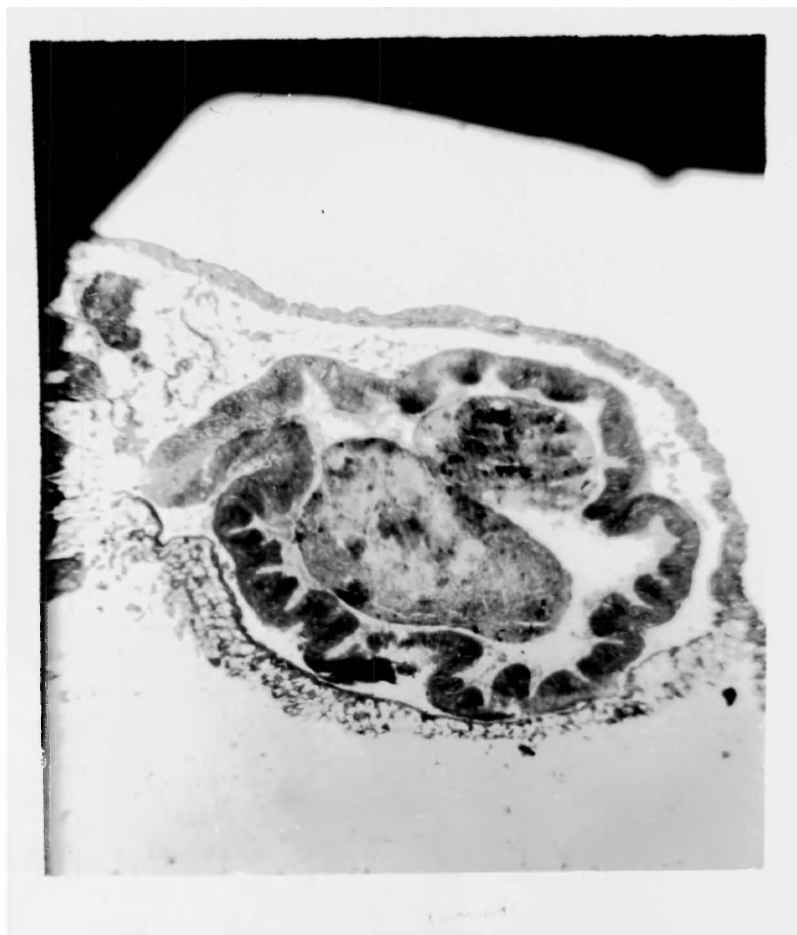


PLATE 22

EXPLANATION OF PLATE

Cross section of the intestine x 950

Section taken through the mid-region of the intestinal tract proper showing the highly compressed columnar cells of the intestinal villi. The appearance is one of pseudo stratification. Cilia in this region of the alimentary tract are longer than in any other part. Nuclei, when basal in position, are ovoid. Toward the free ends of the cells the nuclei are compressed in the direction of the main axes of the cells. Nucleoli are visible in the nuclei. Areas of thin circular muscle are visible along the left margin of the intestine.



PLATE 23

EXPLANATION OF PLATE

Cross section of the rectum x 300

Section taken through the mid-region of the rectum. Visible are the rectal villi, fecal pellets, and cilia. This plate is histologically comparable to that of Plate 21 of the intestine. The villi in this region are not quite as high as those found in the intestinal tract. The diameter of the tract is considerably greater than that of the intestinal tract.



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